DIAGNOSTIC CHARACTERIZATION OF *ANOPHELES FREEBORNI* AND *AN. HERMSI* BY HYBRID CROSSES, FREQUENCIES OF POLYTENE X CHROMOSOMES AND rDNA RESTRICTION ENZYME FRAGMENTS

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ABSTRACT. A polytene chromosome analysis was prepared from *Anopheles freeborni* collected from 25 locations in north and central California, and parts of Washington and Oregon. The X chromosome banding pattern, thought previously to be specific to *An. hermsi*, was common in mosquitoes collected from foothill regions in California, and in all samples from Washington and Oregon. At some of these locations, many mosquitoes had heterokaryotypes for the inversion that distinguishes the X chromosome of *An. freeborni* from that of *An. hermsi*. Use of rDNA restriction site analysis, and the results from crossing of different strains bearing either type of X chromosome, showed that *An. hermsi* does not have a unique or diagnostic X chromosome. *Anopheles hermsi* was collected in San Mateo County, CA, which is now the northernmost known limit of this species. Crossing studies, or the examination of rDNA restriction enzyme profiles, are presently the only means of identifying *An. hermsi*.

INTRODUCTION

A new species of anopheline mosquito, *Anopheles hermsi*, was described recently by Barr and Guptavanij (1988). At present, *An. hermsi* is thought to be limited to areas of southern California south of the Tehachapi Mountains (Cope et al. 1988). This species has been implicated in recent autochthonous cases of vivax malaria in southern California (Maldonado et al. 1990). Prior to 1988, mosquitoes now known as *An. hermsi* were believed to be a coastal strain of *Anopheles freeborni* Aitken (Aitken 1939, 1945), a geographic variant of *Anopheles occidentalis* Dyar and Knab (Lewallen 1957) or a new species (Baker and Kitzmiller 1963, Fujioka 1986,[4] Menchaca 1986[5]).

At present, there are no reliable anatomical characters that distinguish eggs, larvae or adults of *An. hermsi* from those of *An. freeborni*. According to the description of *An. hermsi* (Barr and Guptavanij 1988), the only character that distinguishes both species is a simple inversion on the polytene X chromosome. The banding patterns on the autosomes of both species are either identical (Baker 1965,[6] Fritz 1989[7]) or too similar (Menchaca 1986[5]) to be useful as diagnostic characters. Baker and Kitzmiller (1963) first described the X chromosome of *An. hermsi* (then referred to as "southern occidentalis") and noted that it had unmistaken homology with that of *An. freeborni*. Both types of X chromosome appeared to differ only by a single inversion (In(X)A) at subzones 2B–5C (Baker 1965[6]).

As a part of a study on the population genetics of *An. freeborni*, we examined the polytene chromosomes of specimens collected throughout north and central California, and parts of Oregon, Washington and Utah. The purpose of this cytogenetic survey was to distinguish *An. freeborni* from *An. hermsi*, record chromosomal polymorphisms and use these polymorphisms as genetic markers to determine population structure and species distributions.

Our initial survey showed that the X chromosome banding pattern described as specific to *An. hermsi* was present in some samples collected in northern and central California, and parts of Oregon and Washington. Furthermore,

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[4] Fujioka, K. K. 1986. Hybridization and electrophoretic studies of 3 members of the North American *Anopheles maculipennis* complex (Diptera: Culicidae). Ph.D. dissertation, University of California, Los Angeles.

[5] Menchaca, D. M. 1986. The cytogenetic study of an undescribed member of the North American *Anopheles maculipennis* (Diptera: Culicidae) complex. Ph.D. dissertation, University of California, Los Angeles.

[6] Baker, R. H. 1965. Cytogenetic evidence for the evolutionary relationships among the Nearctic *maculipennis* species of anopheline mosquitoes. Ph.D. dissertation, University of Illinois, Urbana.

[7] Fritz, G. N. 1989. Mass rearing and population genetics of *Anopheles freeborni*. Ph.D. dissertation, University of Florida, Gainesville.

Table 1. Collection site number and location for samples of *Anopheles* collected in California, Oregon and Washington.

Site no.	Location
	California
1	Nevada Co., Wolf Creek & Highway 49
2	El Dorado Co., Camino, Carson Rd.
3	El Dorado Co., Pleasant Valley Rd.
4	Sacramento Co., Sloughouse
5	Sacramento Co., Folsom
6	Sutter Co., Highway 99 & Howsley Rd.
7	Yolo Co., Capay Valley, Guinda
8	Yolo Co., Knights Landing
9	Colusa Co., Millers Landing
10	Colusa Co., Highway 20 near Williams
11	Sutter Co., west of Yuba City on Butte House Rd.
12	Butte Co., Chico
13	Tehama Co., Tehama, Gyle Rd.
14	Glenn Co., east of Willows on Highway 162
15	Lake Co., Clear Lake
16	Sonoma Co., Sonoma, Huichica Cr.
17	Sacramento Co., Highway 99 & Twin Cities Rd.
18	San Mateo Co., Jasper Ridge Preserve on Sand Hill Rd.
19	Kern Co., Onyx, Canebrake Creek
20	Inyo Co., Big Pine
21	Inyo Co., Bishop
	Oregon
22	Jefferson Co., Madras
23	Umatilla Co., Hermiston
	Washington
24	Benton Co., Richland
25	Yakima Co., Yakima

many mosquitoes at some of these locations were heterokaryotypes (one copy of the standard X chromosome specific to *An. freeborni* and one copy of In(X)A). These unexpected results raised some important questions:

1) Was *An. hermsi* more widely distributed than thought previously?

2) Were *An. freeborni* and *An. hermsi* really distinct species?

3) Were individuals of *An. freeborni* and *An. hermsi* mating with one another in sympatric populations?

4) Was one species, both, or perhaps an unidentified third species polymorphic for In(X)A?

To answer these questions, we have crossed various geographic strains having one or the other type of X chromosome. According to Fujioka (1986[4]), crossing *An. freeborni* to *An. hermsi* will produce sterile hybrid male progeny. In addition, we examined rDNA restriction enzyme pattern variations, which were shown recently to distinguish both species (Collins et al. 1990). We report here the frequency and distribution of both types of X chromosomes found in our cytogenetic study, the results obtained from crossing different strains and the results of the rDNA analyses.

MATERIALS AND METHODS

Insect samples and chromosome preparation: Larvae and adults were collected during July–October 1988 from 25 different sites (Table 1, Fig. 1). In California, many areas throughout the Sacramento Valley were sampled as well as locations in the foothills of the Sierra Nevada, the coastal range, 2 locations in the Owens Valley and one location near the coast at Palo Alto. The techniques described by French et al. (1962) were used to prepare the polytene chromosomes. Bloodfed females were kept at 28 °C for approximately 28–29 h. Ovaries were dissected in dilute Carnoy's and then transferred to 75% acetic acid. Chromosomes, banding patterns and inversions were identified using a photographic map made of the best polytene chromosome preparations. This map was used in conjunction with the description and standard map of ovarian nurse-cell polytenes (Faran 1981[8]). The

[8] Faran, T. C. 1981. The adult ovarian nurse cell chromosomes of *Anopheles* (*Anopheles*) *freeborni* Aitken 1939 (Diptera: Culicidae). M.Sc. thesis, University of Maryland, College Park.

Fig. 1. Frequency of the standard (white) and inversion (black) karyotype for the X chromosome at collection sites in California, Oregon and Washington.

Marysville strain (which Faran used to prepare her map) was obtained from the Walter Reed Army Institute of Research and used as a standard for comparing banding patterns.

rDNA probe: The rDNA of *An. freeborni* and *An. hermsi* have a restriction enzyme site difference in the external and internal spacer regions (Collins et al. 1990). These diagnostic differences were used to identify species in this study. In a blind test, the rDNA of at least 3 individuals from each of 12 collection sites in California, Washington and Oregon was probed in the manner described by Collins et al. (1990). These sites included those in which mosquitoes were polymorphic for the inversion on the X chromosome, as well as those fixed for either homokaryotype. Prior to the rDNA analysis, the polytene X chromosome karyotype of each female mosquito was determined. Samples of *An. hermsi* from 3 sites in southern California were also probed.

Crosses: Crosses were made among several geographic strains presumed to be *An. freeborni*. To examine widely separated geographic strains

that also showed some habitat and chromosomal differences, a strain from Richland, WA (WASH strain, site 24, Table 1), was crossed to a strain from Davis (DAVIS strain) in the Sacramento Valley, CA. Mosquitoes in the former site were polymorphic for In(X)A (i.e., included heterokaryotypes), an inversion hitherto unknown in *An. freeborni*. The DAVIS strain possessed the standard homokaryotype only.

Three strains from California, which were fixed for one or the other form of X chromosome, were also crossed. These included: 1) JASP strain (fixed for In(X)A), collected at the Jasper Ridge Preserve (site 18) just west of Palo Alto and ca. 16 km from the coast; 2) LAKE strain (also fixed for In(X)A), collected at the north end of Clear Lake (site 15) in Lake County; and 3) DAVIS strain (standard homokaryotype). When crosses were made, the WASH strain was in its 9th laboratory generation, whereas the LAKE and JASP strains were in their first. The DAVIS strain had already been maintained in the laboratory for over 4 years.

Parental crosses among the JASP, LAKE and DAVIS strains were done by forced copulation (Baker et al. 1962). The parental crosses between the WASH and DAVIS strains and all other crosses were done by combining virgin mosquitoes of the same age and of each sex. These were then allowed to mate freely in cages. Bloodfeeding, handling of eggs and all other rearing techniques were standardized and are described in detail by Fritz (1989[7]). Egg batches that did not hatch were held for at least 6 days and checked for the presence or absence of embryonic development. The spermathecae of females that laid unhatched eggs were dissected to determine the presence or absence of spermatozoa. Uninseminated females that did not lay any eggs ($n = 8$) and those that died without laying eggs ($n = 4$) were excluded from any statistical analyses. At least 10 hybrid males from each cross were checked for the presence of spermatozoa in the testes and for the development of normal genitalia.

All parental crosses and backcrosses were compared with one another and with controls for fertility and fecundity. The polytene chromosomes of hybrid females were also checked for banding pattern homology and the degree of synapsis between homologues.

Basic summary statistics of the data were done using SAS (Statistical Analysis Software). A one-way analysis of variance (ANOVA) compared mean values of the number of eggs laid/female, the percentage of eggs hatched, the number of pupae produced per egg batch, the number of adults emerging and the sex ratio.

RESULTS

X chromosome: In California, all mosquitoes from the Sacramento Valley (sites 4–14 in Table 2, Fig. 1) and the Owens Valley (sites 20, 21) had the standard X chromosome described by Kitzmiller and Baker (1963) and Faran (1981[8]) as that of *An. freeborni*. Samples from the coastal mountain range or the foothills of the Sierra Nevada, however, were either fixed for In(X)A (type of X chromosome found in *An. hermsi*) or were polymorphic for the inversion. At Clear Lake (site 15), for example, 8% of the mosquitoes sampled were heterokaryotypes. At Onyx (site 19), the frequency of heterokaryotypes appeared to be greater, but the sample size was too small for an accurate estimate of karyotype frequency. Jasper Ridge (site 18) and Camino (site 2) were fixed for the inversion karyotype. Another sample taken from Camino (site 3) was unusual in that one standard homokaryotype was found among 12 inversion homokaryotypes.

All mosquitoes collected in Madras, OR (site 22), were inversion homokaryotypes (Table 2, Fig. 1). As one proceeded north into Washington State, the frequency of the standard karyotype increased. At Yakima (site 25) the standard X chromosome karyotype reached its highest frequency of 0.90. The observed frequencies of the homokaryotypes and heterokaryotypes in Hermiston (site 23), Richland (site 24) and Yakima (site 25) did not differ significantly from those expected under Hardy-Weinberg equilibrium (Table 2).

Crosses: In the series of parental crosses between the DAVIS and WASH strains, no significant differences were observed in fecundity, fertility and the percentage of adult emergence (Table 3). The sex ratio (males/females) ranged from 1.07 to 1.48, but none was significantly different ($P>0.05$). Although the 2 reciprocal crosses differed significantly from each other in the mean number of pupae produced, neither cross differed from the controls.

Backcrosses did not differ significantly from controls in fecundity, fertility and percentage of adult emergence. The sex ratios ranged from 0.91 to 1.36, but none differed significantly from each other ($P>0.05$). Ten of 12 backcrosses produced a significantly higher percentage of pupae than controls; in effect, post-hatch mortality was significantly lower among backcrosses. The controls in the backcross series differed significantly from each other in percentage pupation, although this difference was not evident in the controls done during the parental crosses.

Table 2. Observed (o) and expected (e) numbers of standard homokaryotypes (S/S) inversion homokaryotypes (I/I) and heterokaryotypes (S/I) for In(X)A of *Anopheles* collected from various sites in California, Oregon and Washington.

		Chromosome X							Freq.	
		S/S		S/I		I/I		Chi		
Site[a]	*n*	o	e	o	e	o	e	sq.	S	I
1	1	1	—	0	—	0	—	—	1.00	0.00
2ab	27	0	0	0	0	27	0	0	0.00	1.00
3ac	13	1	0.08	0	1.85	12	11.08	12.51	0.08	0.92
4	9	9	9	0	0	0	0	0	1.00	0.00
5	2	2	—	0	—	0	—	—	1.00	0.00
6	50	50	50	0	0	0	0	0	1.00	0.00
7	3	3	—	0	—	0	—	—	1.00	0.00
8	22	22	0	0	0	0	0	0	1.00	0.00
9	35	35	35	0	0	0	0	0	1.00	0.00
10	50	50	50	0	0	0	0	0	1.00	0.00
11	22	22	22	0	0	0	0	0	1.00	0.00
12	27	27	27	0	0	0	0	0	1.00	0.00
13	44	44	44	0	0	0	0	0	1.00	0.00
14	50	50	50	0	0	0	0	0	1.00	0.00
15b	60	0	0.01	5	4.56	55	55.20	0.04	0.04	0.96
16	1	—	—	—	—	1	—	—	0.00	1.00
17	16	16	16	0	0	0	0	0	1.00	0.00
18a	41	0	0	0	0	41	41	0	0.00	1.00
19	4[b]	0	—	3	—	1	—	—	0.38	0.62
20	9	9	9	0	0	0	0	0	1.00	0.00
21	2	2	—	0	—	0	—	—	1.00	0.00
22ab	15	0	0	0	0	15	15	0	0.00	1.00
23c	47	6	3.61	14	18.83	27	24.57	3.06	0.28	0.72
24d	73	26	27.73	38	34.46	9	10.73	0.75	0.62	0.38
25e	50	41	40.50	8	9.00	1	0.50	0.62	0.90	0.10

[a] Sample sites followed by the same letter do not differ significantly in frequencies of S/S, S/I and I/I (homogeneity chi square $P = 0.05$).

[b] Includes 2 females collected by Stan Cope.

The testes of the hybrid F_1 males were similar in appearance and amount of sperm as those of controls, and all genitalia appeared normal. The chromosomes of F_1 hybrids synapsed equally well as those of controls.

In the parental crosses among the LAKE, DAVIS and JASP strains, the controls did not differ significantly from the reciprocal crosses in fecundity, fertility, the percentage of individuals pupating or the percentage of individuals emerging as adults (Table 4). The ratios of males to females ranged from 1.07 to 1.48 but were not significantly different among parental crosses ($P>0.05$).

All F_1 hybrid males from crosses between the LAKE and DAVIS strains had genitalia and quantities of sperm that were similar to those observed in the controls. However, F_1 hybrid males from crosses between the LAKE or DAVIS strain and the JASP strain were completely or partially sterile. When the male parent of the hybrid was from the LAKE or DAVIS strain and the female was from the JASP strain, the hybrid male progeny had no sperm in their testes. Although the genitalia appeared to be normal, the testes were often translucent and smaller than those of the controls. F_1 hybrid males, from crosses in which the female parent was from LAKE or DAVIS, had varying amounts of sperm in their testes. The amount varied from none to quantities that appeared normal; the testes were filled with what appeared to be globular spermatocytes and partially developed spermatozoa.

The results of the backcross series (Table 4) confirmed the results obtained from the dissection of hybrid males. All crosses involving hybrid males, in which the parental male was from the LAKE or DAVIS, produced eggs that did not hatch or contain any stage of embryonic development. Most crosses involving hybrid males, in which the parental male was from the JASP strain, had mean percentage egg hatches that were significantly lower than those of the controls; unhatched eggs in these crosses also contained no embryos.

Table 3. Mean number[a] of eggs laid/female, percent[b] hatch, percent[b] pupation and percent[b] emergence of adults of crosses made between the DAVIS strain (D) and the WASH strain (W) of *Anopheles freeborni*.

Cross[c]	*n*	Eggs/ female	% hatch	% pupation	% emergence
		Experiment 1: Parental crosses and controls			
W × W	17	131a	70.2a	61.3ab	89.3a
D × D	17	135a	78.9a	64.1ab	92.7a
W × D	19	140a	72.4a	51.9b	92.2a
D × W	18	126a	66.3a	74.1a	92.5a
		Experiment 2: Backcrosses and controls			
D × D	10	111ab	95.4a	62.4ef	93.9ab
W × W	9	125ab	96.4a	31.5g	94.9ab
DW × D	10	97b	93.1a	83.1abc	90.3ab
DW × W	10	153a	77.5a	83.2abc	91.3ab
DW × DW	10	125ab	81.4a	81.7abc	89.8ab
D × DW	9	120ab	92.7a	79.5abcd	87.4b
W × DW	10	129ab	90.9a	92.8a	92.2ab
WD × W	9	138ab	92.9a	81.9abc	93.9ab
WD × D	9	148ab	95.0a	83.5abc	94.1ab
WD × WD	10	153a	94.9a	87.0ab	91.3ab
W × WD	10	128ab	84.4a	66.8cdef	92.4ab
D × WD	9	152a	93.7a	75.6abcde	90.7ab
DW × WD	10	93b	93.4a	90.8ab	96.2ab
WD × DW	10	141ab	76.1a	86.9ab	97.4a

[a] Means in the same column and experiment, followed by the same letter, are not significantly different ($P>0.05$). Separate ANOVAS were done for each experiment.

[b] Percentages refer to the proportion of individuals moving from one life stage to the next.

[c] The first letter of each cross represents the female parent.

There were no significant differences between controls and backcrosses in the mean number of eggs laid per female. All hybrid females were fertile and had similar mean percentage hatch as controls when backcrossed to either parental strain. Many of the backcrosses had a mean percentage hatch that was higher than the controls, indicating possible heterosis, but none of these differences were significant ($P>0.05$).

The ovarian polytene chromosomes of hybrid progeny did not differ from controls in the amount or degree of synapsis, or in banding pattern. The X chromosomes of hybrids between the LAKE and JASP strains (both fixed for In(X)A) synapsed completely. All hybrids from crosses between the JASP or LAKE strain with the DAVIS strain (fixed for the standard karyotype) were heterokaryotypes.

rDNA probe: Samples of mosquitoes from 11 of 12 collection sites in California, Washington and Oregon had the same restriction enzyme fragment pattern regardless of their X chromosome karyotype (Table 5). Mosquitoes from Jasper Ridge (site 18), and those from samples of *An. hermsi* collected in southern California, were the only individuals with a restriction fragment pattern specific to *An. hermsi*. No individuals had a restriction pattern that was a hybrid of that found in *An. hermsi* and *An. freeborni*.

DISCUSSION

As yet there are no sibling species of anophelines that, when crossed, do not produce some degree of sterility in the hybrid progeny. This is true even when the species are homosequential in their polytene chromosome banding patterns. For example, *An. atroparvus* Van Thiel and *An. labranchiae* Falleroni are homosequential species in the Palearctic Maculipennis Group and produce sterile male hybrids (Bianchi 1968, Coluzzi and Coluzzi 1969).

The degree and causes of sterility among species vary greatly, but it is generally true that post-zygotic barriers exist between most sibling species of mosquitoes (see reviews by Kitzmiller 1953, Kitzmiller et al. 1967, Kitzmiller 1976, Narang and Seawright 1991). In such crosses eggs may not hatch, or larvae may only reach a certain stage of development; sex ratios can also be skewed, and adults can be malformed or sterile. In crosses where adults are produced, it is almost always the males that are sterile. Males may be sterile in only one of the 2 reciprocal parental crosses or in both. Of the 30 possible crosses between sibling species in the *An. gambiae* complex, all but 2 produce sterile males (Davidson 1964, Davidson and Hunt 1973).

Table 4. Mean number[a] of eggs laid/female, percent[b] hatch, percent[b] pupation and percent[b] emergence of adults for crosses made between the DAVIS (D), LAKE (L) and JASP (J) strains of *Anopheles freeborni*.

Cross[c]	*n*	Eggs/ female	% hatch	% pupation	% emergence
Experiment 1: Parental crosses and controls					
D × D	6	146ab	81.3a	73.9a	91.3a
J × J	9	119ab	64.2a	53.0a	87.4a
L × L	5	118ab	79.6a	59.0a	88.6a
D × L	3	93b	86.1a	73.9a	100.0a
D × J	5	159a	93.2a	80.4a	98.3a
L × D	4	156ab	76.4a	76.8a	87.9a
J × D	9	137ab	93.9a	71.2a	85.9a
J × L	9	126ab	82.2a	67.4a	89.9a
L × J	3	163a	87.5a	56.3a	81.8a

Experiment 2: Backcrosses and controls

Cross[c]	*n*	Eggs/ female	% hatch	Cross[c]	*n*	Eggs/ female	% hatch
D × D	5	94ab	78.59abcd	JD × D	4	134a	99.1a
L × L	4	104ab	70.3abcd	JD × J	6	94ab	62.2cd
J × J	6	100ab	55.1de	L × LD	4	102ab	96.8ab
D × DJ	8	120ab	16.6f	L × DL	5	142a	90.1abc
DJ × DJ	6	142a	25.5ef	LD × L	8	120ab	97.9ab
J × DJ	6	116ab	19.3f	LD × D	4	118ab	98.5ab
J × LJ	3	64b	30.6ef	LD × LD	6	117ab	83.8abcd
L × LJ	3	119ab	3.3f	D × DL	6	122ab	96.9ab
LJ × LJ	6	128ab	10.0f	D × LD	5	98ab	82.1abcd
D × JD	8	104ab	0.0f	DL × L	5	67b	74.7abcd
J × JD	6	118ab	0.0f	DL × DL	4	113ab	96.2abc
JD × JD	4	122ab	0.0f	DL × D	4	141a	76.8abcd
L × JL	4	99ab	0.0f	JL × J	4	107ab	50.5ef
J × JL	4	95ab	0.0f	JL × L	5	105ab	72.7abcd
JL × JL	5	90ab	0.0f	LJ × L	6	78ab	74.6abcd
DJ × D	6	97ab	95.2abc	LJ × J	6	98ab	89.8abc
DJ × J	4	121ab	95.1abc				

[a] Means in the same column, followed by the same letter, are not significantly different ($P > 0.05$). Separate ANOVAS were done for each experiment.

[b] Percentages refer to the proportion of individuals moving from one life stage to the next.

[c] The first letter of each cross represents the female parent.

In our study, sterile hybrid progeny were produced only when the JASP strain was crossed to either the LAKE or DAVIS strain. The cause of sterility was due to a complete lack or small quantity of developed spermatozoa in hybrid males. This pattern is identical to that found by Fujioka (1986[4]) when he crossed *An. hermsi* to *An. freeborni*.

Cytoplasmic incompatibility between strains due to symbionts (Barr 1980) or the movement of transposable elements does not provide a good explanation for the sterility observed in crosses of the JASP strain with the LAKE or DAVIS strain. Cytoplasmic incompatibility, as observed in natural populations of *Culex pipiens* Linn., is maternally inherited and causes sterility in the parental generation; in effect, the parental female deposits an egg raft that generally fails to hatch.

Transposable elements can cause hybrid dysgenesis, which usually occurs when a female from a strain lacking a particular transposon (e.g., P element in *Drosophila*) is crossed to a male from a strain having the transposon. Therefore, dysgenesis occurs generally in just one of the 2 reciprocal cross-hybrids. Hybrid dysgenesis is characterized by substantially elevated rates of mutation, chromosomal rearrangement and illicit recombination in males (*Drosophila*). Dysgenic sterility in *Drosophila* is usually more pronounced in females (Engels 1980).

In our study, as in Fujioka's (1986[4]), sterility was limited to males and was present in hybrids from both reciprocal crosses. Fecundity and fertility of hybrid females was not significantly different from that of controls, suggesting that disruption of germline genetic and developmental integrity (characteristic of hybrid dysgenesis) had not occurred. Consequently, genetic differences between the JASP strain and the LAKE or DAVIS strain, rather than transpos-

Table 5. Sample site, type of polytene X chromosome, and the rDNA probe determination of species. F = *freeborni* type; H = *hermsi* type; HF = heterokaryotype; — = not scored.

Site	X chrom.	rDNA probe	Site	X chrom.	rDNA probe
2	H	F	20	F	F
2	H	F	20	F	F
2	H	F	22	H	F
6	F	F	22	H	F
6	F	F	23	HF	F
6	F	F	23	F	F
12	F	F	23	H	F
12	F	F	24	HF	F
12	F	F	24	F	F
13	F	F	24	H	F
13	F	F	25	HF	F
13	F	F	25	F	F
15	HF	—	25	H	F
15	H	F	1,003*	H	H
15	H	F	1,003	H	H
18	H	H	1,063*	H	H
18	H	H	1,063	H	H
18	H	H	1,063	H	H
19	HF	F	1,074*	H	H
19	—	F	1,074	H	H
19	—	F	1,074	H	H

* Collections of *Anopheles hermsi* from southern California. 1,003: Riverside Co., Rubidoux, Carlson Pk; 1,063: Ventura Co., Piru Creek; 1,074: San Luis Obispo Co., Santa Margarita.

able elements and cytoplasmic incompatibilities, seem to be the cause of sterility in hybrids.

Results from the rDNA probe are consistent with those of the crossing study in assigning only the population at Jasper Ridge (site 18) to *An. hermsi.*

In general, polytene X chromosomes are useful diagnostic characters for distinguishing cryptic species of anopheline taxa (Kitzmiller et al. 1967, Kitzmiller 1977). There are, however, exceptions to the distinctiveness of the X chromosomes. For example, *An. stephensi* Liston and *An. farauti* Laveran have homosequential X chromosomes, and other species are known to differ in the frequency of a common polymorphic inversion (see review by Kitzmiller 1977).

This investigation shows that, contrary to the description by Barr and Guptavanij (1988), the X chromosome found in *An. hermsi* is not unique to this species, since it is also found in mosquitoes throughout the foothill regions of north and central California, and in parts of Oregon and Washington.

It is uncertain whether these foothill mosquitoes should be classified as *An. freeborni* or a new species. In Oregon and Washington, both X chromosome karyotypes are present in Hardy-Weinberg equilibrium. However, in California, Hardy-Weinberg equilibrium with respect to X chromosome karyotype does not appear to be the rule. The collection of one standard homokaryotype among 12 inversion homokaryotypes in site 3 (Table 2), and similar results from recent collections (G. N. Fritz, unpublished data) suggest some degree of reproductive isolation between both X chromosome karyotypes. Since there is no hybrid sterility or rDNA restriction pattern difference between *An. freeborni* collected in the Central and Owens valleys compared with those mosquitoes collected in foothill regions, we are, at present, considering them as simply ecotypes of one species.

The results from this study support the specific designation of *An. hermsi.* Furthermore, *An. hermsi* was not found to be sympatric with or hybridizing with *An. freeborni* at any of the locations sampled in this study. However, since *An. hermsi* does not have a unique X chromosome, the only way to identify this species reliably is by use of a rDNA probe or by crossing studies.

Prior to this study, *An. hermsi* was known only as far north as Santa Maria, San Luis Obispo County (Cope et al. 1988). It is now apparent that this species extends up the California coast as far north as San Mateo County, and probably farther. Bailey et al. (1972), for example, collected *An. freeborni* near San Pablo Bay and along the Russian River near Healdsburg (Sonoma Co.). Since both sites are near the coast, it is possible that these mosquitoes were actually *An. hermsi.*

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